

Focus on TGF- β SignallingPituitary actions of ligands of the TGF- β family: activins and inhibins

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Abstract

Activins, as members of the transforming growth factor- β superfamily, control and orchestrate many physiological processes and are vital for the development, growth and functional integrity of most tissues, including the pituitary. Activins produced by pituitary cells work in conjunction with central, peripheral, and other local factors to influence the function of gonadotropes and maintain a normal reproductive axis. Follistatin, also produced by the pituitary, acts as a local buffer to bind activin and modulate its bioactivity. On the other hand, inhibins of gonadal origin provide an endocrine feedback signal to antagonize activin signaling in cells that express the inhibin co-receptor, betaglycan, such as gonadotropes. This review highlights the pituitary roles of activin and the mechanisms through which these actions are modulated by inhibin and follistatin.

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Introduction

The growth and differentiation factors (GDFs), comprising the transforming growth factor- β (TGF- β) family, control many biological processes by orchestrating and modulating the developmental program, growth and differentiation profile, and functional homeostasis of most cell types. They act as morphogens to regulate asymmetric cell division and cell fate determination during embryogenesis and have profound effects on reproductive function, immune responses, bone formation, liver growth and regeneration, tissue remodeling and repair, erythropoiesis, and angiogenesis throughout adult life (Vale *et al.* 1990, Massagué 2000). In general, members of the TGF- β family are not only viewed as important suppressors of primary tumorigenesis but, remarkably, are also capable of promoting tumor growth depending on circumstances and cellular context (Roberts & Wakefield 2003). Recent studies have revealed that members of the TGF- β family are also involved in regulating pathways that help to maintain embryonic stem cells in an undifferentiated state (Ying *et al.* 2003, Beattie *et al.* 2005). The factors that are represented in this large family of structurally related polypeptide ligands include the activins, inhibins, TGF- β isoforms, bone morphogenetic proteins (BMPs), GDFs, as well as Nodal and Lefty (Vale *et al.* 1990, Massagué

2000). With few exceptions, these protein factors are expressed and secreted near their targets, where they act as autocrine and/or paracrine factors to locally modify diverse cellular functions of those cells that express appropriate receptors. Cellular responses to individual ligands, in turn, are tempered through extracellular mechanisms of receptor antagonism and ligand inactivation, as well as intracellular events that terminate signaling (Harrison *et al.* 2005).

Numerous *in vitro* and *in vivo* animal studies, as well as data from clinical settings have substantiated the importance of activins and inhibins as modulators of the reproductive axis and normal reproductive function (de Kretser *et al.* 2002, Welt *et al.* 2002, Tong *et al.* 2003, Muttukrishna *et al.* 2004, Luisi *et al.* 2005). Inhibins and, shortly thereafter, activins were identified as components of gonadal fluids that exhibit the ability to suppress or stimulate follicle-stimulating hormone (FSH) secretion from pituitary gonadotropes respectively (Vale *et al.* 1990). The characterization of these factors, as members of the TGF- β superfamily, initiated a cascade of novel ideas regarding their role in the regulation of the reproductive axis and expanded our appreciation of the scope of their function. It is now clear that activins and inhibins regulate reproductive function by exerting effects at all levels of the reproductive axis (Welt *et al.*

2002). Thus, not surprisingly, genetic manipulations or mutations that either delete or alter the expression of the relevant ligands of the TGF- β family, their binding proteins or components of their signaling pathways are associated with varying degrees of reproductive anomalies (Chang *et al.* 2002). In the pituitary, as in many other tissues, activins are produced and act locally to regulate the function of their targets, including gonadotropes and other cell types (Bilezikjian *et al.* 2004). Inhibins, on the other hand, have a more established role as endocrine feedback modulators of the pituitary, although several lines of evidence suggest that they also act locally as autocrine or paracrine factors (Welt *et al.* 2002, Kumar *et al.* 2003). In addition to activins and inhibins, some of the TGF- β and BMP isoforms also exert cell-specific effects within the pituitary and are implicated in the regulation of the reproductive axis in some species (Welt *et al.* 2002). Beyond their reported actions on differentiated pituitary cells, the stage-specific opposing gradients of BMP4 and FGF-8 or BMP2 and Wnt4 (Wingless-type MMTV integration site family, member 4) are critical for the invagination and formation of Rathke's pouch and the normal development of the pituitary (Rosenfeld *et al.* 2000). The purification efforts that culminated in the discovery of gonadal activins and inhibins also led to the discovery of the FSH-suppressing activity of the follistatins, later shown to stem from their activin-binding function (Nakamura *et al.* 1990). Further investigations demonstrated the ubiquitous nature of follistatin distribution and established the importance of the local function of follistatins in the modulation of activin and BMP signaling (Nakamura *et al.* 1990). This review summarizes the evidence for the physiologic roles of activins and their two functional antagonists, inhibins and follistatin, in the modulation of gonadotropes.

Signaling mechanisms of activin

With few exceptions, the biologically active ligands of the TGF- β family, including the activins, BMPs, and TGF- β s, are generally homodimers of protein subunits that display a common structural feature shared by the members of the larger family of cystine-knot growth factors (Vale *et al.* 1990, Massague 2000, Vitt *et al.* 2001). Activins are sulfhydryl-linked dimers of inhibin/activin- β subunits (Vale *et al.* 1990). Of the five β -subunits that have been identified to date, homo- or hetero-dimers of β A and β B are the only ones with demonstrated biological function as receptor ligands (Vale *et al.* 1990). The other β -subunits, on the other hand, may have alternative modes of actions (Mellor *et al.* 2003). The ligands of the TGF- β superfamily initiate downstream signaling events by sequentially interacting with cognate type-II and type-I receptors belonging to an extended family of single-transmembrane Ser/Thr kinase proteins (Lebrun *et al.* 1997, Massague 2000). Structural

studies have revealed some of the important features of these interactions and provided evidence for the 'six-chain complex' generated by the assembly of two type-II and two type-I receptors by the dimeric nature of the ligands (Greenwald *et al.* 2004, Lin *et al.* 2006). The assembly of two types of receptors serves an important function to enable the constitutively active kinase domains of the type-II receptors to *trans*-phosphorylate and activate the type-I receptors and, in turn, allow them to phosphorylate downstream-signaling substrates (Lebrun *et al.* 1997, Feng & DALLAERTS *et al.* 2005, Massague *et al.* 2005). Activins initiate downstream-signaling events by sequentially interacting with one of the two known type-II activin receptors, ActRII or ActRIIB, and the type-I receptor known as activin receptor-like kinase (ALK) 4 (Lebrun *et al.* 1997, Massague 2000). Further analysis has suggested that in addition to ALK4, the activin B and activin AB isoforms may preferentially signal via ALK7 in tissues that express this type-I receptor (Tsuchida *et al.* 2004). Interestingly, activins and TGF- β s share the same downstream-signaling substrates (Lebrun *et al.* 1997, ten Dijke *et al.* 2000, Feng & Derynck 2005, Massague *et al.* 2005). The ligand-specific activation of ALK4 by activin or ALK5 by TGF- β facilitates the direct phosphorylation of Smad2 and/or Smad3 by the kinase domain of these receptors and promotes their ability to enter the nucleus and modify target gene activity in co-operation with Smad4/DPC4 (deleted in pancreatic carcinoma) and other partners. The activation of the Smad-signaling pathway by activin or TGF- β also initiates an intracellular mechanism of feedback modulation by inducing the inhibitory Smad7 and preventing further Smad2/3 activation (Lebrun *et al.* 1997, Massague *et al.* 2005).

Modes of inhibin and follistatin action

Inhibins function as potent cell selective antagonists of activins and, under certain circumstances, of certain BMPs (Lewis *et al.* 2000, Wiater & Vale 2003). Inhibin A and inhibin B are formed by the heterodimerization of the corresponding inhibin/activin β -subunits with the structurally related inhibin/activin α -subunit (Vale *et al.* 1990, Welt *et al.* 2002). The molecular basis of inhibin antagonism of either activin or BMP was elucidated by the realization that the type-III TGF- β receptor (T β RIII or betaglycan) has a dual function and serves as a high-affinity co-receptor for inhibin as well as TGF- β (Lewis *et al.* 2000). Binding and functional studies support a model of antagonism in which the interaction of inhibin with betaglycan facilitates the recruitment of ActRII or ActRIIB into a complex and sequesters them away from activins. In a similar manner, inhibin-bound betaglycan is able to antagonize BMP actions that are dependent on ActRII or ActRIIB, as well as BMPRII (Wiater & Vale 2003). The dual co-receptor function of betaglycan, on the other hand, may allow TGF- β to

interfere with the suppressive actions of inhibin and, indirectly, promote activin bioactivity (Ethier *et al.* 2002). Although betaglycan displays high affinity for both inhibin and TGF- β , recent studies have shown that a single mutation within the inhibin-binding extracellular domain of betaglycan disrupts inhibin and TGF- β binding to this site, but does not affect TGF- β binding to the second TGF- β -binding domain, which does not bind inhibin (Wiater *et al.* 2006). Betaglycan mRNA and protein are present in inhibin-responsive cells throughout the hypothalamo-pituitary-gonadal axis including gonadotropes (MacConell *et al.* 2002, Chapman & Woodruff 2003). The broader distribution of betaglycan beyond these known inhibin-responsive sites probably reflects its additional function as a co-receptor for promoting TGF- β 2 action, as well as its unexpected role in facilitating the antagonism of selective BMP actions by inhibin (MacConell *et al.* 2002, Chapman & Woodruff 2003, Wiater & Vale 2003). The functional versatility of betaglycan and the broader actions of inhibin are likely reflected in the phenotypes of mice that are deficient either in inhibin or its co-receptor, betaglycan. In the inhibin-deficient mice, cell-selective perturbations and dysregulated signaling by both activin and BMP may contribute to the formation of gonadal and adrenal tumors of inhibin-deficient mice (Chang *et al.* 2002). In the betaglycan-deficient mice, on the other hand, embryonic lethality due to heart and liver defects could reflect diminished TGF- β 2 and/or unrestrained BMP signaling (Stenvers *et al.* 2003).

Follistatins are recognized as activin-binding proteins that can bio-neutralize and thereby modulate all actions of activins, and, at higher concentrations those of certain BMPs (Michel *et al.* 1993, Phillips & deKretser 1998, Balemans & Van Hul 2002, Shimasaki *et al.* 2004). Although first identified as FSH-suppressing components of gonadal fluids, numerous studies have documented the presence of follistatin in many tissues, including most cell types of the anterior pituitary (Bilezikjian *et al.* 2004). Mutagenesis studies had previously indicated that follistatin interferes with activin function by masking its type-II binding site (Fischer *et al.* 2003). The recent elucidation of the crystal structure of a follistatin:activin complex has extended this model and revealed the manner in which follistatin masks the binding sites on activin for both type-I and type-II receptors (Thompson *et al.* 2005). These structural studies have also provided a model that would explain the basis for the differential binding modes of follistatin to activin and BMP isoforms (Thompson *et al.* 2005). Two follistatin isoforms (FS315 and FS288) that differ in their abilities to associate with cell-surface proteoglycans are encoded by two alternatively spliced mRNA products of the follistatin gene (Michel *et al.* 1990, Schneyer *et al.* 2004). A third isoform (FS303) that has been identified in porcine follicular fluids is generated from the proteolytic cleavage of FS315 (Schneyer *et al.* 2004). The relative

abundance of the various follistatin isoforms has been difficult to evaluate, but recent measurements confirm that FS315 is the form that circulates, while the C-terminally truncated FS288 form is presumed to act locally because of its greater affinity for cell-surface proteoglycans (Welt *et al.* 2002, Keutmann *et al.* 2004, Schneyer *et al.* 2004). Genetic models of altered follistatin expression have established the importance of this modulator in restraining the actions of activins, BMPs, and possibly other related ligands (Chang *et al.* 2002). Mice deficient in follistatin develop embryonic defects and die shortly after birth (Matzuk *et al.* 1995). Follistatin overexpression, on the other hand, is associated with infertility probably resulting from disrupted activin and BMP signaling at the level of both the gonads and the pituitary (Guo *et al.* 1998).

Local pituitary actions of activin

The various components required for activin signaling are present in the anterior pituitary and activins have been reported to exert effects on multiple pituitary cell types, the best-characterized of which are the gonadotropes (Bilezikjian *et al.* 2004). The regulated and the differential production of FSH from pituitary gonadotropes through different stages of the reproductive cycle is achieved by the integrated actions of multiple factors that originate from central, peripheral, or local sources. Activins play a central role in this process and numerous studies have demonstrated that the differential production of FSH is exquisitely dependent on the actions of this TGF- β family member to induce FSH- β synthesis (Vale *et al.* 1990, Bilezikjian *et al.* 2004). Early studies demonstrated that activins exert their effects by differentially stimulating FSH- β mRNA accumulation (Carroll *et al.* 1989). Recent data, however, raise the possibility that activins, under certain circumstances, can also regulate luteinizing hormone (LH) production by modifying the expression of the LH- β -subunit (Yamada *et al.* 2004, Coss *et al.* 2005). Genetic knockout or overexpression models, while provide some answers regarding the function of the activin circuitry in reproductive function, have also highlighted the complexity of the system (Chang *et al.* 2002).

The autocrine or paracrine function of activin in the pituitary became appreciated by the results of a series of studies that confirmed that inhibin/activin α - and β -subunit mRNAs and proteins are expressed in the pituitary and that anterior pituitary cell preparations secrete activins A and B (Bilezikjian *et al.* 2004). Experiments with an immunoneutralizing MAB to activin B subsequently provided convincing evidence that activin B produced by rat pituitary cells serves as a positive signal and locally drives the expression of FSH- β and the secretion of FSH, *in vitro*, and mediates the hypersecretory FSH response to ovariectomy, *in vivo* (Corrigan *et al.* 1991, DePaolo *et al.* 1992). The fact that

pituitaries of ovariectomized rats, when grafted under the kidney capsule, continue to overproduce FSH is consistent with the existence of a local mechanism that operates independent of the hypothalamic and gonadal inputs (DePaolo *et al.* 1992). More recent studies with castrated ActRII null mice have provided additional support for not only a local activin tone, but also an inhibin tone (Kumar *et al.* 2003). Activin B originating from gonadotropes, the primary sites of inhibin/activin α - and β B-subunit expression, works in concert with factors known to modulate this cell type, including gonadotrophin-releasing hormone (GnRH), gonadal steroids, inhibin, and local follistatin, and, in turn, is influenced by them (Bilezikjian *et al.* 2004). Through a paracrine mechanism, activin A originating from other pituitary cell types, including folliculostellate cells, may also participate in modulating the responses of gonadotropes (Bilezikjian *et al.* 2003). Activins are permissive for the actions of GnRH on FSH production and GnRH pulse frequency, in turn, can alter activin B production by modulating inhibin/activin β B mRNA levels (Weiss *et al.* 1992, Burger *et al.* 2002). The availability of suitable cell lines (LPT2 and α T3-1), derived from the gonadotrope lineage of the mouse pituitary, has permitted direct examination of the mechanisms underlying the actions of activin in gonadotropes. Transcriptional studies of FSH- β and GnRH receptor promoters in these cell lines have revealed that both are modulated by activin (Fernandez-Vazquez *et al.* 1996, Duval *et al.* 1999, Pernasetti *et al.* 2001, Norwitz *et al.* 2002, Suszko *et al.* 2003, Bernard 2004) and targets the Smad2/3 pathway used by activin (Pernasetti *et al.* 2001, Norwitz *et al.* 2002, Suszko *et al.* 2003, Bernard 2004). These studies have shown that activin modulates gonadotrope sensitivity to GnRH by facilitating the action of GnRH to promote the transcription of the FSH- β and GnRH receptor genes (Pernasetti *et al.* 2001, Gregory *et al.* 2005). The precise cellular mechanisms used by activins to facilitate the expression of their multiple targets and coordinate gonadotrope responsiveness are not known at this time, but are likely to be achieved through differential partnerships between Smads and other transcription factors that are formed in response to different cellular inputs and sensitive to different thresholds of activin signaling.

Pituitary actions of inhibin

The inhibin/activin α -subunit mRNA and protein are expressed in the anterior pituitary, but the endocrine feedback function of gonadal inhibin seems to be its principal mode of action to differentially modulate FSH production (de Kretser *et al.* 2002, Welt *et al.* 2002). The importance of circulating inhibin has been substantiated by a number of *in vivo* studies using wild-type animals, as well as genetic models. Immunoneutralization of circulating inhibin with an antibody to the inhibin/

activin α -subunit was shown to increase plasma FSH levels and pituitary FSH- β mRNA levels, while injections of recombinant inhibin A had the opposite effect (Vale *et al.* 1990). In inhibin/activin α -subunit knockout mice, liver-specific expression of exogenous inhibin A lowered circulating FSH levels and resulted in reproductive defects, demonstrating that circulating inhibin can reach the pituitary to regulate FSH production (Pierson *et al.* 2000). Gonadotropes are the main pituitary targets of inhibin and the majority of these cells express the inhibin co-receptor, betaglycan (Lewis *et al.* 2000, MacConell *et al.* 2002, Chapman & Woodruff 2003). Inhibins modulate the function of gonadotropes by antagonizing the actions of activins on FSH secretion and FSH- β expression (Bilezikjian *et al.* 2004, Gray *et al.* 2004, Phillips & Woodruff 2004) and by modulating gonadotrope sensitivity to GnRH receptors (Wang *et al.* 1989, Braden *et al.* 1990, Sealfon *et al.* 1990, Gregg *et al.* 1991). Whether the local production of inhibin within the pituitary contributes to the feedback actions of circulating inhibin to modulate the function of gonadotropes or other pituitary cell types remains an open question for future studies (Kumar *et al.* 2003, Bilezikjian *et al.* 2004).

Local pituitary actions of follistatin

Measurable levels of follistatins also circulate, but their endocrine role as feedback modulators of activins in the pituitary remains questionable. Quite a number of studies, on the other hand, has substantiated the importance of autocrine or paracrine function of follistatins as local modulators of the actions of activins in the pituitary and many other tissues (Welt *et al.* 2002, Bilezikjian *et al.* 2004). Most pituitary cell types express follistatin mRNA and cultured rat anterior pituitary cells secrete the protein (Kogawa *et al.* 1991, Kaiser *et al.* 1992, Bilezikjian *et al.* 1993, Lan-Lee *et al.* 1993). The observation that folliculostellate cells isolated from bovine pituitaries secrete substantial amounts of follistatin protein provided one of the first clues regarding the potential role of this protein as an autocrine or paracrine factor of the pituitary (Gospodarowicz & Lau 1989). Folliculostellate cells isolated from rat anterior pituitaries have also been reported to produce large amounts of follistatin (Bilezikjian *et al.* 2003). Experimental manipulation of the local availability of free follistatin in cultured rat anterior pituitary cells later confirmed that the local follistatin tone of the pituitary may be an important buffer that controls the potency and the efficacy of locally secreted activins to drive basal FSH secretion from gonadotropes (Bilezikjian *et al.* 1993, Fischer *et al.* 2003). These observations emphasize the importance of maintaining a delicate balance between activin and follistatin tone to achieve physiologically relevant levels, patterns of FSH production, and prevent reproductive

anomalies due to excessive activin signaling in gonadotropes.

Activins are potent inducers of pituitary follistatin expression and the level of local follistatin expression, to some extent, is determined by the intrapituitary activin B tone, which also drives FSH- β expression (Bilezikjian *et al.* 1993). These actions of activins, however, are self-limiting and sensitive to the intrinsic follistatin tone through a reciprocal feedback loop (Bilezikjian *et al.* 2004). A substantial portion of pituitary follistatin is likely to originate from gonadotropes and, therefore, is subject to regulation by factors that influence this cell type including GnRH, the autocrine action of activin B, inhibins, and gonadal steroids. Experimental data suggest that this may be an important feature underlying the ability of these factors to control follistatin production and thereby exert an indirect control on the actions of activin on gonadotropes. For example, a number of studies has suggested that GnRH and gonadal factors exert their effects on FSH production in part by modulating follistatin and inhibin/activin β B subunit expression in the pituitary and thus altering the local availability and ratio of activin B and follistatin (DePaolo *et al.* 1993, Besecke *et al.* 1996, Dalkin *et al.* 1998). Moreover, differential FSH and LH production may be achieved, in part, through the differential effects of different patterns of GnRH pulses on inhibin/activin β B and follistatin expression (Kirk *et al.* 1994, Dalkin *et al.* 1999). Rapid GnRH frequencies have been shown to support maximal follistatin expression with no change in FSH- β , while slower frequencies produce a selective rise in FSH- β , but no change in follistatin (Kirk *et al.* 1994). Moreover, the fluctuations in pituitary follistatin mRNA levels may partly account for the rise and fall in FSH production across the reproductive cycle (Halvorson *et al.* 1994, Bauer-Dantoin *et al.* 1996, Besecke *et al.* 1997).

Follistatin production from the folliculostellate cells provides another level of control on the actions of activins in the pituitary. The exact function of these S100 positive non-endocrine cells of the pituitary is not fully appreciated yet. They have been reported to be a major source of several paracrine factors of the pituitary to form a network that facilitates the synchronization of the pituitary gland or to represent a group of progenitor cells with the potential to differentiate into specialized endocrine cells (Allaerts *et al.* 1990, Renner *et al.* 1996, Stojilkovic 2001, Inoue *et al.* 2002). Folliculostellate cell lines derived from rat, mouse, and human anterior pituitaries have been characterized in recent years and experiments with these cells are beginning to shed some light into the function of this cell type (Inoue *et al.* 1992, Danila *et al.* 2000b, Bilezikjian *et al.* 2003). As a major source of intrapituitary follistatin, they have an important function to exert local control on the pituitary actions of activin (Gospodarowicz & Lau 1989, Bilezikjian *et al.* 2003). Follistatin production from folliculostellate cells is negatively correlated with

FSH- β expression and a paracrine modulator of FSH secretion (Fuji *et al.* 2002, Kawakami *et al.* 2002, Bilezikjian *et al.* 2003). Recently, characterized rat anterior pituitary folliculostellate cells (FS/D1h) have provided some useful and surprising information. These cells produce substantial amounts of follistatin (Bilezikjian *et al.* 2003). They express activin receptors and are responsive to activin as measured by the induction of Smad7 mRNA levels and inhibition of proliferation (Bilezikjian *et al.* 2003). Surprisingly, activin has no effect on follistatin mRNA levels in FS/D1h, unlike its effects on gonadotropes (Bilezikjian *et al.* 1999, 2003). The FS/D1h cells, however, respond to interleukin-1 β (IL-1 β) and dexamethasone by a dramatic increase in follistatin production both at the mRNA and protein level (Bilezikjian *et al.* 2003). Both activin A and follistatin have been implicated as participants of the systemic inflammatory response and the evidence suggests that the dramatic rise in circulating follistatin may be secondary to cytokine-induced increases in activin A (Phillips *et al.* 2001). Because FS/D1h cells also express inhibin/activin β A mRNA and, therefore, might secrete some activin A, the effect of IL-1 β on follistatin production could have also been indirectly mediated by an autocrine action of activin A. An inhibitor of ALK4,5,7 (a generous gift from John Saunders; Neurocrine Biosciences, Inc., La Jolla, CA, USA), which suppresses signaling in response to activins and other ligands that utilize these type-I receptors, was used to address this possibility (Inman *et al.* 2002). These experiments indicated that unlike the case seen in

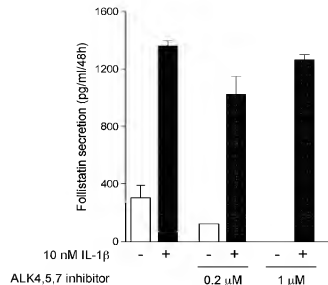


Figure 1 Follistatin secretion from FS/D1h folliculostellate cells in response to interleukin-1 β (IL-1 β) is not affected by co-incubation with an inhibitor of ALK4,5,7 to block activin signaling. Follistatin levels were quantified using an indirect immunoassay that measures follistatin:[¹²⁵I]activin A complexes that are immunoprecipitated with anti-FS288 (#5542), as previously described (Bilezikjian *et al.* 2003).

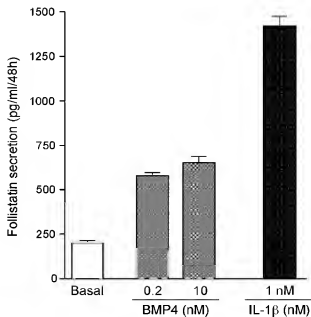


Figure 2 The dose-dependent effects of BMP4 and IL-1 β on follistatin secretion from FS/D1h folliculostellate cells. Follistatin measurements were performed as previously described (Bilezikjian *et al.* 2003).

systemic inflammation, cytokine-induced follistatin production from FS/D1h cells was probably not mediated by activins (Fig. 1). Both IL-1 β and glucocorticoids have been reported to modulate FSH production from gonadotropes (Bohnsack *et al.* 2000, Leal *et al.* 2003). The observations that both these agents can modulate follistatin production from FS/D1h cells suggest that their actions on FSH may, in part, be mediated by the paracrine action of follistatin derived primarily from folliculostellate cells. Interestingly, recent observations raise the possibility that folliculostellate cells may also be targets of BMPs, as indicated by the dose-dependent effects of BMP4 on follistatin secretion from FS/D1h cells (Fig. 2). The significance of this BMP4 effect remains to be evaluated, but could reflect another mode of

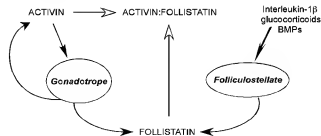


Figure 3 A model of the autocrine and paracrine actions of pituitary follistatin. In this model, activin modulates its own bioactivity by an autocrine mechanism, promoting follistatin production from gonadotropes and preventing further signaling. Follistatin secretion from folliculostellate cells in response to agents, such as IL-1 β , glucocorticoids, BMP4, and possibly other BMP isoforms modulates activin bioactivity by a paracrine mechanism.

paracrine control of the pituitary. Altogether, these observations have revealed the existence of diverse mechanisms through which cell-selective inputs influence the intrapituitary follistatin tone and modify activin signaling within the pituitary by autocrine or paracrine mechanisms (Fig. 3).

Conclusions

The activin and follistatin circuitries of the pituitary have a critical function to regulate the differential production of FSH from gonadotropes throughout the reproductive cycle. The collective findings of numerous studies provide strong evidence that activin exerts these effects by an autocrine mechanism, which itself is subject to multiple levels of modulation involving the intracellular feedback mechanism of Smad7 (Bilezikjian *et al.* 2001), the endocrine feedback actions of inhibin (de Kreter *et al.* 2002, Welt *et al.* 2002), and the autocrine and paracrine actions of follistatin (Bilezikjian *et al.* 2004). Beyond the established importance of this local circuitry in the regulation of gonadotropes and possibly other cell types, the precise control of the local activin:follistatin balance may also be a critical checkpoint for the control of pathogenic mechanisms that lead to pituitary cell proliferation and tumor formation (Danila *et al.* 2000a, Ezzat 2001, Risbridger *et al.* 2001). Although significant progress has been made in unraveling these complex processes, significant challenges involving the identification of cell-specific mechanisms that can be precisely manipulated or targeted for therapeutic benefits still remain.

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